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(54) **CEA derivatives lacking the Hydrophobic C-terminal domain.**

(57) The present invention provides recombinant CEA glycoprotein derivatives and to methods for their production. These recombinant CEA glycoprotein derivatives are characterized in that they are free from cross-reactive CEA-like antigens, antigenically indistingiushable from the soluble form of CEA shed from tumor cells and devoid of ethanolamine. Said derivatives preferably have the amino acid sequence [SEQ ID NO: 1]. The said CEA glycoprotein derivatives may be used as reagents in an immunoassay for the diagnosis of neoplastic diseases. The invention also relates to a DNA encoding a said recombinant CEA glycoprotein derivative such as the DNA having the nucleotide sequence [SEQ ID NO: 2] or a functional equivalent sequence thereof. The present invention also relates to recombinant vectors comprising a said DNA, which recombinant vector is capable of directing the expression of the said DNA in a compatible host cell and to transformed host cells containing such a recombinant vector. The recombinant CEA glycoprotein derivative of the present invention, preferably integrated in a test-kit may be used for determining the presence of tumor cells in a sample of body fluid.

EP 0 618 292 A1

The present invention relates to derivatives of the carcinoembryonic antigen (CEA).

Carcinoembryonic antigen (CEA) is one of the most studied human tumor markers and is widely used in the diagnosis of neoplastic diseases, such as colorectal cancer. Thus, e.g. when the serum levels of CEA are elevated in a patient, a drop of CEA levels after surgery means that the resection of the tumor was successful. On the other hand a subsequent rise in serum CEA levels after surgery indicates that metastases of the original tumor may have formed or that new primary tumors have grown. For a review see Shively J. E. and Beatty J. D., "CEA-related antigens: molecular biology and clinical significance", *Crit. Rev. Oncol. Hematol.* **2**, 355-399 [1985]; and Mach J. -P., Pèlegrin A. and Buchegger F., "Imaging and therapy with monoclonal antibodies in non-hematopoietic tumors", *Curr. Opin. Immunol.* **3**, 685-693 [1991].

The complete cDNA sequence encoding the CEA protein codes for a polypeptide of 702 amino acids consisting of a 34 amino acid residues long leader peptide, a 108 amino acid residues long NH_2-terminal domain, three homologous repeating domains of 178 amino acid residues and a hydrophobic C-terminal domain of 26 amino acid residues (Zimmermann W., Ortlieb B., Friedrich R. and von Kleist S., "Isolation and characterization of cdna clones encoding the human carcinoembryonic antigen reveal a highly conserved repeating structure", *Proc. Natl. Acad. Sci. U S A* **84**, 2960-2964 [1987]; Beauchemin N., Benchimol S., Cournoyer D., Fuks A. and Stanners C. P. "Isolation and characterization of full-length functional cDNA clones for human carcinoembryonic antigen", *Mol. Cell. Biol.* **7**, 3221-3230 [1987]; Oikawa S., Kosaki G. and Nakazato H., "Molecular cloning of a gene for a member of carcinoembryonic antigen (CEA) gene family; signal peptide and N-terminal domain sequences of nonspecific crossreacting antigen (NCA)", *Biochem. Biophys. Res. Commun.* **146**, 464-469 [1987]). The 34 amino acid residues long leader peptide is cleaved from the precursor CEA polypeptide in the process of the transfer through the endoplasmatic reticulum membrane.

The hydrophobic C-terminal domain is also missing in the mature membrane-bound CEA glycoprotein. It has been shown that CEA is attached to the membrane through a phosphatidylinositol-glycan tail (PI-G) covalently linked through ethanolamine to the COOH-terminal residue of mature CEA (Hefta S. A., Hefta L. J., Lee T. D., Paxton R. J. and Shively J. E., "Carcinoembryonic antigen is anchored to membranes by covalent attachment to a glycosylphosphatidylinositol moiety: identification of the ethanolamine linkage site", *Proc. Natl. Acad. Sci. U S. A.* **85**, 4648-4652 [1988]). It is generally assumed that CEA is processed post-translationally to remove the hydrophobic C-terminal domain with subsequent addition of a PI-G anchor to the last amino acid of the third repeated domain.The PI-G tail can be cleaved by phosphatidylinositol-specific phospholipase C releasing the membrane-bound form of CEA. The soluble form of CEA formed in this way always comprises the ethanolamine residue coupled to the carboxy-terminus of the last amino acid sequence present in the mature form of CEA (i.e. the carboxy-terminus of the last amino acid of the third repeated domain) and possibly some fragment of the phosphatidylinositol-glycan tail.

It has been suggested (Caras I. W., Weddell G. N., Davitz M. A., Nussenzweig V. and Martin D. W.,Jr., "Signal for attachment of a phospholipid membrane anchor in decay accelerating factor", *Science.* **238**, 1280-1283 [1987]; Hefta L. J., Schrewe H., Thompson J. A., Oikawa S., Nakazato H. and Shively J. E., "Expression of complementary DNA and genomic clones for carcinoembryonic antigen and nonspecific cross-reacting antigen in Chinese hamster ovary and mouse fibroblast cells and characterization of the membrane-expressed products", *Cancer Res.* **50**, 2397-2403 [1990]; Hemperly J. J., Edelman G. M. and Cunningham B. A. "cDNA clones of the neural cell adhesion molecule (N-CAM) lacking a membrane-spanning region consistent with evidence for membrane attachment via a phosphatidylinositol intermediate", *Proc. Natl. Acad. Sci. U S A* **83**, 9822-9826 [1986]) that the COOH-terminal domain of PI-G anchored proteins is important for their correct targeting and attachment to the cell surface. Complete or partial deletion of the hydrophobic domain can result in the secretion of mutant proteins into the medium (Udenfriend S., Micanovic R. and Kodukula K., "Structural requirements of a nascent protein for processing to a PI-G anchored form: studies in intact cells and cell-free systems", *Cell Biol. Int. Rep.* **15**, 739-759 [1991]).

The standard CEA reference presently used is generally isolated from extracts of human tumors. This CEA appears to be shed from the cell surface of tumors by cleavage of the PI-G anchor (Kuroki M., Murakami M., Wakisaka M., Ikeda S., Oikawa S., Oshima T., Nakazato H., Kosaki G. and Matsuoka Y., "Immunoreactivity of recombinant carcinoembryonic antigen proteins expressed in Escherichia coli", *Immunol. Invest.* **21**, 241-257 [1992]). The disadvantage of the CEA isolated from human tumor extracts is that it may contain cross-reactive CEA-like antigens which may interfere with an immunoassay of the CEA released by tumors. It is known that these CEA-like antigens are elevated in many non-cancerous conditions, such as e.g. in inflammatory liver diseases and in smokers. Efforts to overcome the problem caused by interfering CEA-like antigens lead to the cloning of the DNA encoding CEA. In EP-A-263,933 various nucleic acid sequences coding for CEA peptid sequences are disclosed.

Efforts to express different domains of the CEA molecule in bacteria showed that the CEA domains expressed in bacteria had a lower antigenicity, presumably due to incomplete folding (Kuroki M., Murakami M., Wakisaka M., Krop Watorek A., Oikawa S., Nakazato H., Kosaki G. and Matsuoka Y., "Epitopes predominantly retained on the carcinoembryonic antigen molecules in plasma of patients with malignant tumors but not on those in plasma of normal individuals", *Jpn. J. Cancer Res.* **83**, 505-514 [1992]).

As an alternative to the use of human tumor extracts, it has been proposed to purify CEA from the culture medium from human cancer cell lines. It has been found however, that the complete mature form of CEA is not actively secreted, but only shed in low amounts (see below) in the culture medium of colon cancer carcinoma cell lines. One solution to overcome the problem of the low-level expression was to prepare fragments of CEA. However, quite obviously the fragments often do not comprise all important epitopes which are present in CEA, i.e. the epitopes generally known as GOLD 1 - 5 described by Hammarstrom et al. (Hammarstrom S., Shively J. E., Paxton R. J., Beatty B. G., Larson A., Ghosh R., Bormer O., Buchegger F., Mach J. -P., Burtin P., Seguin P., Darbouret B., Degorce F., Sertour J., Jolu J. -P., Fuks A., Kalthoff H., Schmiegel W., Arndt R., Kloppel G., von Kleist S., Grunert F., Schwarz K., Matsuoka Y., Kuroki M., Wagener C., Weber T., Yachi A., Imai K., Hishikawa N. and Tsujisaki M. "Antigenic sites in carcinoembryonic antigen", *Cancer Res.* **49**, 4852-4858 [1989]).

The problem to be solved by the present invention was therefore to provide a CEA derivative which is free from cross-reactive CEA-like antigens, is antigenically indistingiushable from the soluble form of CEA shed from tumor cells, i.e. comprises all important epitopes of the CEA protein and on the other hand is secreted in high amounts by a recombinant host.

It has now been found that a recombinant cDNA encoding CEA which lacks the 3' region encoding the 26 amino acid hydrophobic domain is capable of secreting a CEA derivative which fulfils the requirements outlined above. Upon transfection of the said cDNA into a suitable host cell, such as a rat or a human carcinoma cell, a 50- to 100-fold higher level of secretion of a fully immunogenic CEA glycoprotein into the culture medium is obtained.

Therefore, the present invention relates to a recombinant CEA glycoprotein derivative which is characterized in that it is:

(a) free from cross-reactive CEA-like antigens;

(b) antigenically indistinguishable from the soluble form of CEA shed from tumor cells; and

(c) secreted in high amounts in devoid of ethanolamine.

Said recombinant CEA glycoprotein derivative is secreted in high amounts in culture medium from cells transfected with said recombinant cDNA.

The term "free from cross-reactive CEA-like antigens" relates to the fact that the recombinant CEA glycoprotein derivative of the present invention is secreted by host cells transformed with a recombinant vector comprising a cDNA encoding said recombinant CEA glycoprotein derivative. Because it is not isolated from tumor extracts it does not contain the cross-reactive CEA-like antigens usually present in such extracts.

The term "antigenically indistingiushable from the soluble form of CEA shed from tumor cells" means that the recombinant CEA glycoprotein derivative is immunologically the same as the natural form of CEA, i.e. comprises all major epitopes present in CEA, especially that it comprises all five epitopes generally known as the GOLD 1 - 5 epitopes (Hammarstrom et al. [1989], supra).

The term "being devoid of ethanolamine" relates to the fact that the recombinant CEA glycoprotein of the present invention lacks the ethanolamine residue and possibly some fragment of the phosphatidylinositol-glycan tail normally present in the soluble form of CEA as it is obtainable from untransfected tumor cells, e.g. after sheding from tumor cells or alter treatment with a phosphatidylinositol-specific phospholipase. Because the cDNA encoding the recombinant CEA glycoprotein of the present invention lacks the sequence coding for the hydrophobic C-terminal tail present the in the precursor form of CEA polypeptide, the polypeptide expressed from this cDNA cannot be anchored in the cell membrane. It was found that quite surprisingly the lack of the hydrophobic domain in the CEA glycoprotein does not affect the transport of CEA towards the cell surface in either human or rat carcinoma cells, but only prevents its anchoring to the cell surface.

The preferred recombinant CEA glycoprotein derivative of the present invention has the following amino acid sequence:

KLTIESTPFN VAEGKEVLLL VHNLPQHLFG YSWYKGERVD GNRQIIGYVI

GTQQATPGPA YSGREIIYPN ASLLIQNIIQ NDTGFYTLHV IKSDLVNEEA

TGQFRVYPEL PKPSISSNNS KPVEDKDAVA FTCEPETQDA TYLWWVNNQS

LPVSPRLQLS NGNRTLTLFN VTRNDTASYK CETQNPVSAR RSDSVILNVL

YGPDAPTISP LNTSYRSGEN LNLSCHAASN PPAQYSWFVN GTFQQSTQEL

FIPNITVNNS GSYTCQAHNS DTGLNRTTVT TITVYAEPPK PFITSNNSNP

VEDEDAVALT CEPEIQNTTY LWWVNNQSLP VSPRLQLSND NRTLTLLSVT

RNDVGPYECG IQNELSVDHS DPVILNVLYG PDDPTISPSY TYYRPGVNLS

LSCHAASNPP AQYSWLIDGN IQQHTQELFI SNITEKNSGL YTCQANNSAS

GHSRTTVKTI TVSAELPKPS ISSNNSKPVE DKDAVAFTCE PEAQNTTYLW

WVNGQSLPVS PRLQLSNGNR TLTLFNVTRN DARAYVCGIQ NSVSANRSDP

VTLDVLYGPD TPIISPPDSS YLSGANLNLS CHSASNPSPQ YSWRINGIPQ

QHTQVLFIAK ITPNNNGTYA CFVSNLATGR NNSIVKSITV SA **[SEQ ID NO: 1]**

The present invention also relates to functional equivalent recombinant CEA glycoprotein derivatives having an amino acid sequence which is related to the above amino acid sequence by deletions, insertions or substitutions without essentially changing the biological and immunological properties of the said CEA glycoprotein.

Examples of amino acid substitutions which do not substantially alter the biological and immunological properties of a protein have been described, e.g., by Neurath et al., in "The Proteins", Academic Press, New York (1979), in particular in Fig. 6 at page 14 thereof. The most frequently observed amino acid substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val Ala/Glu, Asp/Gly, and vice versa.

The recombinant CEA glycoprotein of the present invention may be labeled by one of the labels known in the art, e.g. by a dye, a radioactive, an enzymatic, a fluorescent or a chemiluminescent label. Preferred label is radioactively labeled iodine (^{125}I).

The recombinant CEA glycoprotein of the present invention may be used as a standard in an immunoassay for detecting CEA in a biological sample, e.g. a sample of a body fluid. The person skilled in the art is in a position to configure such an immunoassay based on the general knowledge in the field of immuno diagnostics. The use of an enzyme linked immuoassay (ELISA) is preferred. Examples for such immunoassays are described e.g. in EP-A-346,710. Enzymes used to label the recombinant CEA glycoprotein derivative of the present invention include, among others alkaline phosphatase, β-galactosidase, horseradish peroxidase, glucose-6-phosphat dehydrogenase, 3-phosphoglycerate kinase (PGK).

The present invention also relates to immunoassays for the diagnosis of neoplastic diseases in which immunoassays a recombinant CEA glycoprotein derivative of the present invention is used, preferably as a standard in form of a reagent, wherein said recombinant CEA glycoprotein derivative is mixed with an inert

carrier material. Examples for such inert carrier materials are distilled water, buffers, possibly containing stabilizers and other additives generally used in reagents for diagnostic purposes.

The present invention also relates to methods for the preparation of such reagents and to the reagents per se, as well as to test-kits for the determination of the presence of cancer cells in a biological sample. Such a test-kit comprises in a container a recombinant CEA glycoprotein derivative in accordance with the present invention, if necessary in combination with an inert carrier material and, if necessary, additional reagents such as e.g. monoclonal or polyclonal CEA antibodies.

The recombinant CEA glycoprotein of the present invention is preferably encoded by a cDNA fragment which lacks the region coding for the C-terminal hydrophobic tail. One way to obtain such a cDNA fragment is to use suitable restriction endonucleases to cut a cDNA encoding the complete form of CEA directly upstream from the region coding for the hydrophobic tail that lacks in the recombinant CEA glycoprotein of the present invention. The 3' end of the cDNA fragment is then restored by using a synthetic oligonucleotide duplex which encodes the extra nucleotides which were inadvertently cleaved of by the restriction endonuclease. Preferably said synthetic oligonucleotide duplex also comprises a stop codon, which causes that the translation is terminated after the last amino acid residue in the amino acid sequence [SEQ ID NO: 1]. The restriction endonuclease EaeI is the most suitable enzyme for preparing the said cDNA fragment. Unfortunately this restriction endonuclease cleaves the the cDNA encoding CEA at more than one position. In order to overcome this problem a 800 base pair fragment comprising the 3' end of the CEA cDNA can be isolated by digestion with Bsu36I and XbaI endonucleases, and then cutting the fragment obtained in this way with EaeI separately.

Thus, the present invention provides also a DNA encoding a CEA glycoprotein derivative of the present invention, such as the DNA having the nucleotide sequence

ATGGAGTCTC CCTCGGCCCC TCCCCACAGA TGGTGCATCC CCTGGCAGAG

GCTCCTGCTC ACAGCCTCAC TTCTAACCTT CTGGAACCCG CCCACCACTG

CCAAGCTCAC TATTGAATCC ACGCCGTTCA ATGTCGCAGA GGGGAAGGAG

GTGCTTCTAC TTGTCCACAA TCTGCCCCAG CATCTTTTTG GCTACAGCTG

GTACAAAGGT GAAAGAGTGG ATGGCAACCG TCAAATTATA GGATATGTAA

TAGGAACTCA ACAAGCTACC CCAGGGCCCG CATACAGTGG TCGAGAGATA

ATATACCCCA ATGCATCCCT GCTGATCCAG AACATCATCC AGAATGACAC

AGGATTCTAC ACCCTACACG TCATAAAGTC AGATCTTGTG AATGAAGAAG

CAACTGGCCA GTTCCGGGTA TACCCGGAGC TGCCCAAGCC CTCCATCTCC

AGCAACAACT CCAAACCCGT GGAGGACAAG GATGCTGTGG CCTTCACCTG

TGAACCTGAG ACTCAGGACG CAACCTACCT GTGGTGGGTA AACAATCAGA

GCCTCCCGGT CAGTCCCAGG CTGCAGCTGT CCAATGGCAA CAGGACCCTC

ACTCTATTCA ATGTCACAAG AAATGACACA GCAAGCTACA AATGTGAAAC

CCAGAACCCA GTGAGTGCCA GGCGCAGTGA TTCAGTCATC CTGAATGTCC

TCTATGGCCC GGATGCCCCC ACCATTTCCC CTCTAAACAC ATCTTACAGA

TCAGGGGAAA ATCTGAACCT CTCCTGCCAC GCAGCCTCTA ACCCACCTGC

ACAGTACTCT TGGTTTGTCA ATGGGACTTT CCAGCAATCC ACCCAAGAGC

TCTTTATCCC CAACATCACT GTGAATAATA GTGGATCCTA TACGTGCCAA

GCCCATAACT CAGACACTGG CCTCAATAGG ACCACAGTCA CGACGATCAC

AGTCTATGCA GAGCCACCCA AACCCTTCAT CACCAGCAAC AACTCCAACC

CCGTGGAGGA TGAGGATGCT GTAGCCTTAA CCTGTGAACC TGAGATTCAG

AACACAACCT ACCTGTGGTG GGTAAATAAT CAGAGCCTCC CGGTCAGTCC

CAGGCTGCAG CTGTCCAATG ACAACAGGAC CCTCACTCTA CTCAGTGTCA

CAAGGAATGA TGTAGGACCC TATGAGTGTG GAATCCAGAA CGAATTAAGT

GTTGACCACA GCGACCCAGT CATCCTGAAT GTCCTCTATG GCCCAGACGA

CCCCACCATT TCCCCCTCAT ACACCTATTA CCGTCCAGGG GTGAACCTCA

GCCTCTCCTG CCATGCAGCC TCTAACCCAC CTGCACAGTA TTCTTGGCTG

ATTGATGGGA ACATCCAGCA ACACACACAA GAGCTCTTTA TCTCCAACAT

CACTGAGAAG AACAGCGGAC TCTATACCTG CCAGGCCAAT AACTCAGCCA

GTGGCCACAG CAGGACTACA GTCAAGACAA TCACAGTCTC TGCGGAGCTG

CCCAAGCCCT CCATCTCCAG CAACAACTCC AAACCCGTGG AGGACAAGGA

TGCTGTGGCC TTCACCTGTG AACCTGAGGC TCAGAACACA ACCTACCTGT

GGTGGGTAAA TGGTCAGAGC CTCCCAGTCA GTCCCAGGCT GCAGCTGTCC

AATGGCAACA GGACCCTCAC TCTATTCAAT GTCACAAGAA ATGACGCAAG

AGCCTATGTA TGTGGAATCC AGAACTCAGT GAGTGCAAAC CGCAGTGACC

CAGTCACCCT GGATGTCCTC TATGGGCCGG ACACCCCCAT CATTTCCCCC

CCAGACTCGT CTTACCTTTC GGGAGCGAAC CTCAACCTCT CCTGCCACTC

GGCCTCTAAC CCATCCCCGC AGTATTCTTG GCGTATCAAT GGGATACCGC

AGCAACACAC ACAAGTTCTC TTTATCGCCA AAATCACGCC AAATAATAAC

GGGACCTATG CCTGTTTTGT CTCTAACTTG GCTACTGGCC GCAATAATTC

CATAGTCAAG AGCATCACAG TCTCTGCATA G [SEQ ID NO: 2].

It is understood that the above DNA sequence includes the codons coding for the 34 amino acid residue long signal peptide which is also present in the natural form of the gene encoding CEA, which signal peptide sequence is cleaved off during the maturation of the CEA protein. On the other hand, quite obviously, said DNA sequence does not comprise the codons encoding the C-terminal hydrophobic domain present in the natural form of the gene encoding CEA.

As indicated above a DNA having the sequence [SEQ ID NO: 2] can be prepared by using the methods of recombinant DNA technology from a cDNA coding for the full-lenght form of CEA. Such a DNA sequence can also be prepared by chemical synthesis and/or by combining appropriate DNA fragments in such a way that a complete DNA having the sequence of [SEQ ID NO: 2] is obtained.

Because of the degeneracy of the genetic code, it will be understood that there are many potential nucleotide sequences (functional equivalents) that could code for the recombinant CEA glycoprotein derivative having the amino acid sequence [SEQ ID NO: 1]. Therefore, the present invention also relates to a functional equivalent sequence of [SEQ ID NO: 2], which nucleotide sequence encodes a functional equivalent CEA glycoprotein derivative as referred to above. Such a functional equivalent nucleotide sequence may readily be prepared using appropriate synthetic oligonucleotides in primer-directed site-specific mutagenesis on the exemplary cDNA of this invention [SEQ ID NO: 2], as described by Morinaga Y., Franceschini T., Inouye S. and Inouye M., "Improvement of oligonucleotide-directed site-specific mutagenesis using double-stranded plasmid DNA", *Bio/Technology* **2**, 636-639 [1984].

The present invention still further provides recombinant vectors containing and capable of directing the expression of a DNA encoding a recombinant CEA glycoprotein derivative in a compatible host cell, and host cells containing such vectors. It should also be understood that the nucleotide sequences of the present invention which are to be inserted into a recombinant vectors may include additional nucleotides, which additional nucleotides are not part of the actual structural gene encoding the recombinant CEA glycoprotein derivative of the present invention, as long as the recombinant vectors containing such sequence or fragments are still capable of directing the production of a recombinant CEA glycoprotein derivative in accordance with the present invention in an appropriate host cell.

The insertion of a DNA encoding a recombinant CEA glycoprotein derivative in accordance with the present invention into a cloning vector is easily accomplished when both the DNA and the desired cloning vector have been cut with the same restriction enzyme or enzymes, since complementary DNA termini are thereby produced. If this cannot be accomplished, it may be necessary to modify the cut ends that are produced by digesting back single-stranded DNA to produce blunt ends, or by achieving the same result by filling in the single-stranded termini with an appropriate DNA polymerase. In this way, blunt-end ligation with an enzyme such as T4 DNA ligase may be carried out. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini. Such linkers may comprise specific oligonucleotide sequences that encode restriction site recognition sequences. The cleaved vector and the DNA coding for the recombinant CEA glycoprotein derivative of the present invention may also be modified by homopolymeric tailing (see Morrow J.F., "Recombinant DNA Techniques", Methods in Enzymology **68**, 3-24 [1979]).

Many of the cloning vectors known to the person skilled in the art may be used for preparing the recombinant vectors in accordance with the present invention. Such cloning vectors comprise one or more marker activities that may be used to select for desired transformants. Examples for such marker activities are e.g. neomycin resistance (see Example below), geneticin resistance and methothrexate resistance.

It should be understood that there are many ways to insert a DNA encoding the recombinant CEA glycoprotein derivative of the present invention into a cloning vector. What is essential in this respect is that the recombinant vector is capable of directing the production of the recombinant CEA glycoprotein derivative in an appropriate host cell.

The recombinant vectors comprising a DNA having a nucleotide sequence encoding a recombinant CEA glycoprotein derivative of the present invention may be prepared by:

(a) inserting a DNA having a nucleotide sequence encoding the recombinant CEA glycoprotein derivative into a vector;

(b) replicating the said vector in a host cell; and

(c) isolating the recombinant vector from the host cell.

The selection of an appropriate host cell is affected by a number of factors known in the art. These factors include, for example, compatibility with the chosen vector, toxicity of proteins encoded by the recombinant vectors, capability to secrete the desired protein, ease of recovery of the desired CEA glycoprotein, expression characteristics, biosafety and costs. A balance of these factors must be considered, and it must be understood that not all hosts will be equally effective for expression of a particular recombinant DNA molecule. The preferred host cells for producing the recombinant CEA glycoprotein of the

present invention are CEA-negative cells. Examples for such cells are human or rat colon carcinoma cells, e.g. the subclone CO115$^-$ from the human colon carcinoma cell line CO115 described by Mach et al. (Mach J. -P., Cartel S., Merenda C., Sordat B. and Cerottini J.-C., "In vivo localization of radiolabelled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice", *Nature* **248**, 704-706 [1974]; see also Carrel S., Sordat B. and Merenda C., "Establishment of a cell line (Co-115) from a human colon carcinoma transplanted into nude mice", *Cancer Res.* **36**, 3978-3984 [1976]) and the rat colon carcinoma cell line PROb described by Caignard et al. (Caignard A., Martin M. S., Michel M. F. and Martin F., "Interaction between two cellular subpopulations of a rat colonic carcinoma when inoculated to the syngeneic host", *Int. J. Cancer* **36**, 273-279 [1985]). The said subclone CO115$^-$ was shown by fluorescent activated cell sorting using a panel of directly fluorescinated anti-CEA monoclonal antibodies, to express no CEA and no CEA-crossreactive antigens at the cell surface. It was also shown by enzyme linked immunoadsorbent assay that the culture medium from the untransfected CO115$^-$ subclone contains no CEA or CEA-crossreacting antigens which may interfere in the assay.

Various methods for introducing a foreign DNA fragment into a cell are known to the person skilled in the art. Examples for such methods are microinjection, electroporation, transfection and infection with a viral vector. The preferred method for inserting a recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention into a cell is the well-known calcium phosphate method (originally described by Graham F.L. and Van der Eb A.J., "A new technique for the assay of infectivity of human adenovirus 5 DNA", *Virology* **54**, 456-467 [1973]). Geneticin-resistant transfectants can be screened for CEA secretion in the supernatant by an ELISA test using anti-CEA antibodies. Once a transformed host cell is produced, the cell population may be amplified in culture a culture medium comprising the necessary nutrients under conditions suitable for the growth of the cell population and/or under conditions suitable for high secretion of the recombinant DNA leading to the production of high amounts of the recombinant CEA glycoprotein derivative of the present invention.

The secreted recombinant CEA glycoprotein derivative of the present invention is secreted into the cell culture medium from which it can be isolated by first removing the cells and cellular debris by low speed centrifugation. The supernatant containing the recombinant CEA glycoprotein derivative of the present invention obtained in this way may then be concentrated by ultrafiltration. Initial separation of the recombinant CEA glycoprotein derivative from the supernatant may be performed by precipitation with salts such as sodium or ammonium sulfate, by ultrafiltration or by other methods well known to those skilled in the art. Further purification can be accomplished by conventional protein purification techniques including but not limited to gel filtration, ion-exchange chromatography, preparative disc-gel or curtain electrophoresis, isoelectric focusing, low temperature organic solvent fractionation, or countercurrent distribution. Purification can also be carried out by immunoaffinity chromatography.

Thus, the present invention provides also a method for producing the recombinant CEA glycoprotein derivative defined above, which method comprises:

(a) culturing a host cell containing a recombinant vector comprising a DNA having a nucleotide sequence encoding the said recombinant CEA glycoprotein derivative, such as the DNA having the nucleotide sequence [SEQ ID NO: 2] or an equivalent sequence thereof, under conditions in which the DNA is expressed; and

(b) isolating the recombinant CEA glycoprotein derivative produced by the host cell from the culture.

The preferred method for the construction of the recombinant cDNA encoding the CEA fragment of the present invention can be summarized as follows:

- In the first step, a suitable vector comprising a cDNA encoding the full length CEA protein, such as the Bluescript™ vector described in the Example below, is digested at a unique site with the Bsu36I endonuclease. The cleaved vector is then dephosphorylated and further digested at a unique site with the XbaI endonuclease to generate two fragments of 0.8 and 4.6 Kb (1 Kb = 1'000 base pairs) which are isolated.
- In the second step, the 4.6 Kb fragment, containing vector DNA and the 5' part of the CEA cDNA, is ligated into a XbaI site with a synthetic oligonucleotide duplex containing the last 43 base pairs (b.p.) of the third CEA repeat downstream from the EaeI site to the codon of the last amino acid found in the mature protein and further containing a TAG stop codon and a XbaI sticky end.
- The third step consists of cleaving the 0.8 Kb fragment referred to above at its unique EaeI site. This resulted in two fragments of about 0.4 Kb each, one fragment consisting of the missing part of the third CEA repeat which was to be retained in the final recombinant cDNA and the other fragment consisting of the hydrophobic tail which should be deleted.
- In the fourth step the vector is circularized. Of the two 0.4 Kb fragments obtained in the previous step, only the one containing the third CEA repeat has the proper sticky ends, viz. Bsu36I and XbaI, for the

double ligation and circularization of the 4.6 Kb fragment joined to the synthetic duplex mentioned above. The resulting construct may be amplified in a suitable vector such as in a Bluescript™ vector. The correct construction of the vector can be checked by restriction analysis using StyI and EaeI endonucleases. The recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention can then be recloned into a vector suitable for the expression of a cDNA in a eukaryotic cell An example for such a vector is the eukaryotic expression vector pRc/CMV referred to in the Example below. The vector pRc/CMV is designed for high level stable expression of inserted genes under the control of the constitutive CMV promoter. The vector comprises also the bovine growth hormone polyadenylation signal and a neomycin resistance gene which is expressed from the SV40 early promoter. The correctness of the final construct can be verified by restriction analysis. The correctness of the new 3' end of the recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention can be confirmed by sequencing.

The vector comprising the recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention and the neomycin-resistance gene are preferably introduced by using the calcium phosphate method transfection method into a CEA-negative cell, such as e.g. the subclone from the human colon carcinoma cell line CO115 (Mach J. -P. et al. [1974], supra; Carrel S. et al. [1976], supra) or the rat colon carcinoma cell line PROb (Caignard A. et al. [1985], supra).

Depending on the host cell used for inserting the recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention, the transformed host cells were found to shed about 50 to 100 times more CEA than host cells transformed with a cDNA encoding the full length CEA protein. Thus, e.g. the subclones from the human colon carcinoma cell line CO115 transformed with the recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention were found to secrete about 7.7 to 13.6 micrograms CEA/10^6 cells/72 h. Under the same conditions, non-transfected human colon carcinoma cells known to have a high level of CEA expression shed about 50 to 300 times less CEA. More precisely the cell line CO112 (Mach J.P. et al. [1974], supra) shed about 0.045 micrograms CEA/10^6 cells/72 h and the cell line LS174T (Rutzky L.P., Kaye C.I., Siciliano M.J., Chao M. and Kahan B.D., "Longitudinal karyotype and genetic signature analysis of cultured human colon adenocarcinoma cell lines LS180 and LS174T", *Cancer Res.* **40**, 1443-1448 [1980]) shed 0.128 micrograms CEA/10^6 cells/72 h).

The PROb rat carcinoma clones transfected with the recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention secreted from 0.61 to 0.99 micrograms CEA/10^6 cells/72 h. Selected clones from the same PROb cells transfected with full-length CEA-cDNA shed a maximum of only 0.015 micrograms CEA/10^6 cells/72 h (Pèlegrin A., Terskikh A., Hayoz D., Chalandon Y., Olsson N. O., Folli S., Buchegger F., Kromer B., Schwarz K., Martin M., Martin F. and Mach J. -P., "Human carcinoembryonic antigen cDNA expressed in rat carcinoma cells can function as target antigen for tumor localization of antibodies in nude rats and as rejection antigen in syngeneic rats", *Int. J. Cancer* **52**, 110-119 [1992]). Thus, the transfection of rat colon carcinoma PROb with a CEA-cDNA lacking the C-terminal domain resulted in a 50 fold higher level of CEA secretion compared to clones from the same cell line transfected with full-length CEA cDNA.

The above results show that there is a difference between the amount of recombinant CEA glycoprotein derivative of the present invention (rCEA) secreted by human cells and rat cells. It has been proposed earlier that both transcriptional and post-transcriptional control mechanisms regulate CEA gene expression in colon carcinomas (Hauck W. and Stanners C. P., "Control of carcinoembryonic antigen gene family expression in a differentiating colon carcinoma cell line, Caco-2", *Cancer Res.* **51**, 3526-3533 [1991]). In view of the identity of the DNA constructs used for transfection this lower secretion rate of rCEA may be attributed to the differences in post-transcriptional control between the two species.

The size of the CEA expressed by the transfected cells can be analyzed by Western blotting (Towbin, H., Staehelin, T. and Gordon J., "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications", *Proc. Natl. Acad. Sci.* USA **76**, 4350-4354 [1979]). Such an analysis is performed as follows: Cell culture supernatants are electrophoretically separated on a SDS-polyacrylamide gel, preferably a 7.5-15% linear gradient SDS-polyacrylamide gel. Then an immunoblot is performed using antibodies recognizing a major epitope on the CEA protein. Preferably a pool of ^{125}I-labeled anti-CEA monoclonal antibodies (MAbs) is used for such purpose. In a typical example the rCEA cDNA-transfected-CO115 human colon carcinoma exemplified below produced a rCEA of about 200 kDa. This corresponds to the size of the CEA protein produced by human colon carcinoma LS174T. When transfected into the PROb rat colon carcinoma, the same rCEA cDNA produced a rCEA with a lower molecular weight (viz. about 144 kDa) as did the full-length CEA cDNA transfected into the PROb rat carcinoma cells (Pèlegrin et al. [1992], supra).

Epitope characterization of ^{125}I-labeled, purified rCEA produced by the host cells transformed with the recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention can be performed by testing the binding of the said rCEA to different anti-CEA MAbs coupled to Sepharose™. In typical experiments using human colon carcinoma CO115 cells transformed with a recombinant cDNA encoding the recombinant CEA glycoprotein derivative of the present invention the five MAbs GOLD 1 to 5 directed against the 5 major epitopes on the CEA molecule (Hammarstrom et al. [1989], supra), binding values ranging from 65% to 88% were found (see Example below). Such binding values compare favorably with those obtained with ^{125}I-labeled CEA purified from a human tumor.

Thus the present invention shows that transfection into human and rat carcinoma cells of a recombinant CEA cDNA clone from which the region coding for the hydrophobic C-terminal domain has been deleted, results in an abundant secretion of fully antigenic rCEA molecules into the medium. As mentioned above CEA is normally anchored to the cell membrane by a phosphatidylinositol-glycan (PI-G) and is only shed into the medium of cultured cells or in the serum of carcinoma patients probably after the cleavage from the membrane by PI-specific phospholipases. The recombinant CEA glycoprotein derivative of the present invention is lacking the C-terminal hydrophobic tail. Therefore, it cannot be PI-G anchored and is directly secreted into the extracellular space. It was found that the lack of the hydrophobic domain in the CEA glycoprotein does not affect the transport of CEA towards the cell surface in either human or rat carcinomas, but only prevents its anchoring to the cell surface. The recombinant CEA glycoprotein derivative of the present invention is recognized by the five well characterized epitopes GOLD 1 - 5.

A Western blot analysis has shown, that surprisingly the rCEA secreted from the transfected rat colon carcinoma has a lower molecular weight (about 144 kDa) than a reference CEA isolated from a human colon carcinoma (about 200 kDa). This is in agreement with the observation that the CEA produced by rat colon carcinoma cells transfected with full-length CEA cDNA has also the same "lower" molecular weight after cleavage by PI-PLC (Pèlegrin et al. [1992], supra). CEA molecules with abnormal molecular weights expressed in heterologous cells transfected with a full length CEA gene have been observed in the case of mouse L-cells and chinese hamster ovary cells (Hefta et al. 1990). The molecules identified on L-cells transfected with total human DNA had a lower molecular weight (150 kDa) than those identified on transfected hamster cells (180 kDa). Incomplete glycosylation seems to be responsible for the smaller molecular weight of the CEA molecules expressed by rat colon carcinoma cells transfected with either full length CEA cDNA or truncated CEA cDNA.

The present invention can be more readily understood by reference to the following Example and the Figures the contents of which Figures are as follows:

Figure 1: Schematic outline of the construction of an exemplary recombinant truncated CEA cDNA in accordance with the present invention. The black box represents the deleted hydrophobic domain. Phosphorylated ends are marked by ℙ.

Figure 2: CEA secretion by different clones and control cell lines. CO115, a CEA-negative clone of a human colon carcinoma cell line; 2C2, 1D6, 2B12, truncated CEA-cDNA CO115 derived transfectants; CO112, LS174T, high CEA-expressing human colon carcinoma cell lines; PROb, a rat colon carcinoma cell line; 1H5, 1A8, 1G7, truncated CEA-cDNA PROb derived transfectants; 3G7/2C11, full-length CEA-cDNA PROb derived transfectants (Pèlegrin et al. [1991], supra).

Figure 3: Western blot analysis of CEA from different clones and control cell lines. Cell culture supernatants were run either directly for the CEA-secreting clones or after treatment of the cells with PI-PLC for the other cells. LS174T, CEA-expressing human colon carcinoma cell line; CO115, CEA-negative clone of human colon carcinoma cell line; 2C2, 1D6, 2B12, truncated CEA-cDNA CO115 derived transfectants; PROb, a rat colon carcinoma cell line; 3G7/2C11, full-length CEA-cDNA PROb derived transfectants (Pèlegrin et al. [1992], supra); 1H5, 1A8, 1G7, truncated CEA-cDNA PROb derived transfectants.

Figure 4: Binding of ^{125}I-control CEA (□) and ^{125}I-rCEA (■) to immobilized MAbs directed against the different CEA epitopes. About 5 ng CEA were incubated for 16 hours at 25 °C with 5 micrograms of each of the 5 anti-CEA MAbs covalently coupled to Sepharose™.

EXAMPLE

Unless otherwise specified, percentages given below for solids in solid mixtures, liquids in liquids, and solids in liquids are on a wt/wt, vol/vol and wt/vol basis, respectively. Furthermore, unless otherwise specified, the suppliers of reagents including the full-length CEA cDNA, as well as the instruments mentioned below are not meant to be mandatory. The skilled person is in a position to select similar

reagents or instruments from other suppliers.

Deletion of the region coding for the hydrophobic tail of CEA

The Bluescript™ (KS+) vector containing full-length CEA cDNA between unique HindIII and XbaI sites (Zimmermann W., Weber B., Ortlieb B., Rudert F., Schempp W., Fiebig H., Shively J. E., von Kleist S. and Thompson J. A., "Chromosomal localization of the carcinoembryonic antigen gene family and differential expression in various tumors", *Cancer Res.* **48**, 2550-2554 [1988]; Pèlegrin et al. [1992], supra)) was digested with endonuclease Bsu36I (Boehringer, Mannheim, Germany) and dephosphorylated with alkaline phosphatase (Boehringer). A second digestion with XbaI (Pharmacia, Uppsala, Sweden) produced two fragments of 0.8 Kb and 4.6 Kb which were separated and electroeluted from a 1% agarose gel (see Fig 1, step 1).

Two oligonucleotides (46 b. each), encoding both strands of the last **43** basepairs of the third CEA repeat, viz. JPM1

5'- GGCCGCAATAATTCCATAGTCAAGAGCATCACAGTCTCTGCATAGT - 3' [SEQ ID NO: 3]

and JPM2

5'-CTAGACTATGCAGAGACTGTGATGCTCTTGACTATGGAATTATTGC - 3' [SEQ ID NO: 4],

respectively were synthesized on a commercial DNA synthesizer. The oligonucleotides were purified by passage through a Sephadex™ NAP-25 column (Pharmacia), lyophylized and dissolved in 10 mM Tris-HCl buffer pH 7.6 containing 0.1 mM EDTA.

After gel purification and annealing, the synthetic oligonucleotides form a synthetic duplex encompassing the 43 b.p. of the third CEA repeat downstream from the EaeI to the codon of the last amino acid found in the mature protein. This was followed by a TAG stop codon and by a XbaI sticky end, which forms the 3' end, while the 5' end had a EaeI sticky end.

The duplex was ligated into the XbaI site with the large 4.6 Kb fragment by overnight incubation at 8°C in 1mM ATP (see Fig.1, step 2). The reaction mixture was separated on a 1% agarose gel in order to purify the 4.6 fragment ligated to the synthetic duplex from the excess of the free duplex form.

The 0.8 Kb fragment was digested with EaeI (Boehringer) (see Fig.1, step 3) and the DNA mixture was ligated with the 4.6 Kb fragment joined to the synthetic duplex (see Fig.1, step 4). After phosphorylation of the Bsu36I site with polynucleotide kinase 4 (Pharmacia), the construct was circularized and transfected into the E.coli strain XL1-blue (Stratagene Cloning Systems, La Jolla, California). Individual clones were analyzed by restriction with StyI and EaeI endonucleases. DNA from selected clones was amplified and isolated. Recombinant CEA cDNA was cut out by double digestion with HindIII and XbaI endonucleases and cloned into a pRc/CMV expression vector (Invitrogene, San Diego, California).

Individual clones were further controlled with the restriction endonucleases StyI and EaeI. DNA from selected clones was amplified, isolated and about 200 nucleotides from the 3' end of the recombinant CEA cDNA, including the 46 b.p. region formed by the synthetic duplex, were sequenced using T7 and SP6 primer with a USB Sequencing kit (USB, Cleveland, Ohio).

Cell cultures and transfections

The human colon carcinoma cell line CO115 was established according to known procedures (Mach et al. [1974], supra; Carrel et al. [1976], supra). The rat colon carcinoma cell line DHD/K12/TRb, referred to as PROb, is a selected subclone derived from a cell line established from a transplantable colon adenocarcinoma induced by 1,2-dimethylhydrazine in a syngeneic BDIX rat (Martin F., Caignard A., Jeannin J. F., Leclerc A. and Martin M., "Selection by trypsin of two sublines of rat colon cancer cells forming progressive or regressive tumors", *Int. J. Cancer.* **32**, 623-627 [1983]). The PROb subclone has been shown to induce progressive tumors in rats of the Berlin Druckrey IX/Orl (BDIX) strain (Caignard et al. [1985], supra). The human and rat cell lines were maintained in RPMI 1640 and Dulbecco F12 medium, respectively,

supplemented with 10% fetal calf serum (FCS). Three micrograms of DNA were precipitated with calcium phosphate (Mammalian Transfection Kit, Stratagene, La Jolla, California) and incubated for 16 hours with about 3 x 10^5 nonconfluent adherent carcinoma cells in 10 ml of culture medium with 10% FCS. The medium was removed and 10 ml of fresh culture medium were then added. After a further 24-hours incubation, the cells were harvested, distributed into four 96-well microtiter plates and grown for 24 hours before adding the neomycin analog G418 (Gibco, Paisley, Scotland) at a concentration of 200 micrograms/ml. The supernatants from each well were screened by ELISA (Buchegger F., Mettraux C., Accolla R. S., Carrel S. and Mach J. -P., "Sandwich enzyme immunoassay using three monoclonal antibodies against different epitopes of carcinoembryonic antigen (CEA)", *Immunol. Lett.* **5**, 85-91 [1982]) for CEA secretion.

Monoclonal antibodies

MAb B93, 35, B17, and CE25 are specific for CEA; they do not bind to crossreacting antigens nor to granulocytes (Buchegger F., Pèlegrin A., Delaloye B., Bischof-Delaloye A. and Mach J. -P., "^{131}-I labeled F-(ab')2 fragments are more efficient and less toxic than intact anti-CEA antibodies in radioimmunotherapy of large human colon carcinoma grafted in nude mice", *J. Nucl. Med.* **31**, 1035-1044 [1984]). MAb 192 is an anti-CEA antibody which cross-reacts with non-specific crossreacting antigen (NCA) (Buchegger F., Schreyer M., Carrel S. and Mach J. -P., "Monoclonal antibodies identify a CEA crossreacting antigen of 95 kD (NCA-95) distinct in antigenicity and tissue distribution from the previously described NCA of 55 kD", *Int. J. Cancer* **33**, 643-649 [1984]). Each of the five Mabs (B93, 35, B17, CE25 and 192) reacts specifically with one of the recently identified Gold 1-5 epitopes of the CEA molecule (Hammarstrom et al. [1989], supra).

Assay for CEA production

An equal number of cells (5x10^5) from CEA cDNA transfected human or rat carcinoma clones or from untransfected human colon carcinomas was added to each well of a 24-well culture plate (Falcon, Becton Dickinson, Oxnard, USA) in 10% FCS RPMI medium. After 18 hours, the complete medium was replaced by 1 ml serum-free medium, which markedly decreases cell proliferation without inhibiting CEA secretion. The supernatants were harvested following a further 72 hours incubation. The amount of CEA in the supernatants was determined by an enzyme linked immunoabsorbant assay (ELISA) using 3 anti-CEA MAbs (Buchegger et al. [1982], supra).

CEA purification and labeling with ^{125}I

rCEA was affinity purified from serum-free culture supernatant on an immunoabsorbant column consisting of MAb B17 coupled to Sepharose™. Batches of 50 ml serum-free culture supernatant were applied at a rate of 2 ml/hour on a 2 ml Sepharose™ column containing 4 mg of B17 MAb. The bound CEA was eluted from the column with 3 M ammonium thiocyanate in H_2O and immediately dialyzed against 0.1 M Tris buffer, pH 7.4.

CEA was extracted from liver metastases using the perchloric acid method (Krupey J., Wilson T., Freedman S. O. and Gold P., "The preparation of purified carcinoembryonic antigen of the human digestive system from large quantities of tumour tissue", *Immunochem.* **9**, 617-622 [1972]; Fritsche R. and Mach J. P., "Isolation and characterization of carcinoembryonic antigen (CEA) extracted from normal human colon mucosa", *Immunochem.* **14**, 119-127 [1977]). Briefly, one volume of tissue was first homogenized in 3 volumes of 0.03 M phosphate buffer, pH 7.0 at 4 °C for 10 minutes in a Sorvall Omnimixer (Sorvall, Newton, CT, USA) at 8,000 rpm. The crude homogenate was extracted with 0.6 M perchloric acid for 20 minutes and centrifuged at 10,000 rpm for 10 minutes. The supernatant was dialyzed against deionized water, lyophilyzed, dissolved in Tris buffer and purified by gel filtration on a Sephadex™ G-200 column followed by a Sepharose™ 6B column.

Batches of 20 micrograms purified CEA and rCEA were labeled with 1 mCi ^{125}I using the chloramine T method. The iodine incorporation was about 30-40%. ^{125}I-labeled CEA and rCEA were further purified by gel filtration on a Sephadex™ G-200 column.

Epitone characterization

The different epitopes of the CEA molecule were analyzed in a direct binding assay. About 5 ng ^{125}I-CEA were incubated for 16 hours at 25 °C with 5 micrograms of each of the 5 anti-CEA MAbs coupled to

CNBr-Sepharose™ (Pharmacia). The percentage of specific binding was determined by measuring the radioactivity bound to the MAb. The non-specific binding of ^{125}I-CEA was determined by similar incubation with an irrelevant IgG coupled to Sepharose™.

Western blot analysis

Cell culture supernatants from selected transfected clones secreting rCEA were analyzed by Western blot analysis without further treatment. Control untransfected human colon carcinoma cells (5-10 x 10^6) synthesizing membrane-bound CEA were treated with 0.5-1.0 unit of phosphatidylinositol-specific phospholipase C (PI-PLC) (Boehringer Mannheim, Germany) for 1 hour at 37°C in RPMI medium containing 1 mg/ml BSA and 20 mM EDTA.

Samples of cell culture supernatants containing about 100 ng CEA were run on a 7.5-15% linear gradient SDS-PAGE gel and transferred to a nitrocellulose membrane (Millipore, Bedford, MA). Biotinylated SDS-PAGE standards (Bio-Rad, Richmond, CA) were used to determine molecular weights. Membranes were incubated overnight with a pool of 4 ^{125}I-labeled anti-CEA MAbs (35, CE25, B93 and B17) and ^{125}I-labeled avidin at 4°C and then subjected to autoradiography.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(B) STREET: Grenzacherstrasse 124
(C) CITY: Basel
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(ii) TITLE OF INVENTION: CEA Derivatives

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 93810214.2
(B) FILING DATE: 25-MAR-1993

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 642 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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1				5				10						15	

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Val Leu Leu Leu Val His Asn Leu Pro Gln His Leu Phe Gly Tyr Ser
                20                  25                  30

Trp Tyr Lys Gly Glu Arg Val Asp Gly Asn Arg Gln Ile Ile Gly Tyr
            35                  40                  45

Val Ile Gly Thr Gln Gln Ala Thr Pro Gly Pro Ala Tyr Ser Gly Arg
        50                  55                  60

Glu Ile Ile Tyr Pro Asn Ala Ser Leu Leu Ile Gln Asn Ile Ile Gln
65                  70                  75                  80

Asn Asp Thr Gly Phe Tyr Thr Leu His Val Ile Lys Ser Asp Leu Val
                85                  90                  95

Asn Glu Glu Ala Thr Gly Gln Phe Arg Val Tyr Pro Glu Leu Pro Lys
            100                 105                 110

Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro Val Glu Asp Lys Asp Ala
        115                 120                 125

Val Ala Phe Thr Cys Glu Pro Glu Thr Gln Asp Ala Thr Tyr Leu Trp
    130                 135                 140

Trp Val Asn Asn Gln Ser Leu Pro Val Ser Pro Arg Leu Gln Leu Ser
145                 150                 155                 160

Asn Gly Asn Arg Thr Leu Thr Leu Phe Asn Val Thr Arg Asn Asp Thr
                165                 170                 175

Ala Ser Tyr Lys Cys Glu Thr Gln Asn Pro Val Ser Ala Arg Arg Ser
            180                 185                 190

Asp Ser Val Ile Leu Asn Val Leu Tyr Gly Pro Asp Ala Pro Thr Ile
        195                 200                 205

Ser Pro Leu Asn Thr Ser Tyr Arg Ser Gly Glu Asn Leu Asn Leu Ser
    210                 215                 220

Cys His Ala Ala Ser Asn Pro Pro Ala Gln Tyr Ser Trp Phe Val Asn
225                 230                 235                 240

Gly Thr Phe Gln Gln Ser Thr Gln Glu Leu Phe Ile Pro Asn Ile Thr
                245                 250                 255

Val Asn Asn Ser Gly Ser Tyr Thr Cys Gln Ala His Asn Ser Asp Thr
            260                 265                 270

Gly Leu Asn Arg Thr Thr Val Thr Thr Ile Thr Val Tyr Ala Glu Pro
        275                 280                 285

Pro Lys Pro Phe Ile Thr Ser Asn Asn Ser Asn Pro Val Glu Asp Glu
    290                 295                 300
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Asp Ala Val Ala Leu Thr Cys Glu Pro Glu Ile Gln Asn Thr Thr Tyr
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Leu Trp Trp Val Asn Asn Gln Ser Leu Pro Val Ser Pro Arg Leu Gln
                325                 330                 335

Leu Ser Asn Asp Asn Arg Thr Leu Thr Leu Leu Ser Val Thr Arg Asn
            340                 345                 350

Asp Val Gly Pro Tyr Glu Cys Gly Ile Gln Asn Glu Leu Ser Val Asp
        355                 360                 365

His Ser Asp Pro Val Ile Leu Asn Val Leu Tyr Gly Pro Asp Asp Pro
    370                 375                 380

Thr Ile Ser Pro Ser Tyr Thr Tyr Tyr Arg Pro Gly Val Asn Leu Ser
385                 390                 395                 400

Leu Ser Cys His Ala Ala Ser Asn Pro Pro Ala Gln Tyr Ser Trp Leu
                405                 410                 415

Ile Asp Gly Asn Ile Gln Gln His Thr Gln Glu Leu Phe Ile Ser Asn
            420                 425                 430

Ile Thr Glu Lys Asn Ser Gly Leu Tyr Thr Cys Gln Ala Asn Asn Ser
        435                 440                 445

Ala Ser Gly His Ser Arg Thr Thr Val Lys Thr Ile Thr Val Ser Ala
    450                 455                 460

Glu Leu Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro Val Glu
465                 470                 475                 480

Asp Lys Asp Ala Val Ala Phe Thr Cys Glu Pro Glu Ala Gln Asn Thr
                485                 490                 495

Thr Tyr Leu Trp Trp Val Asn Gly Gln Ser Leu Pro Val Ser Pro Arg
            500                 505                 510

Leu Gln Leu Ser Asn Gly Asn Arg Thr Leu Thr Leu Phe Asn Val Thr
        515                 520                 525

Arg Asn Asp Ala Arg Ala Tyr Val Cys Gly Ile Gln Asn Ser Val Ser
    530                 535                 540

Ala Asn Arg Ser Asp Pro Val Thr Leu Asp Val Leu Tyr Gly Pro Asp
545                 550                 555                 560

Thr Pro Ile Ile Ser Pro Pro Asp Ser Ser Tyr Leu Ser Gly Ala Asn
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Leu Asn Leu Ser Cys His Ser Ala Ser Asn Pro Ser Pro Gln Tyr Ser
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Ala Lys Ile Thr Pro Asn Asn Asn Gly Thr Tyr Ala Cys Phe Val Ser
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Asn Leu Ala Thr Gly Arg Asn Asn Ser Ile Val Lys Ser Ile Thr Val
625 630 635 640

Ser Ala

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2031 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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ACGCCGTTCA ATGTCGCAGA GGGGAAGGAG GTGCTTCTAC TTGTCCACAA TCTGCCCCAG 180

CATCTTTTTG GCTACAGCTG GTACAAAGGT GAAAGAGTGG ATGGCAACCG TCAAATTATA 240

GGATATGTAA TAGGAACTCA ACAAGCTACC CCAGGGCCCG CATACAGTGG TCGAGAGATA 300

ATATACCCCA ATGCATCCCT GCTGATCCAG AACATCATCC AGAATGACAC AGGATTCTAC 360

ACCCTACACG TCATAAAGTC AGATCTTGTG AATGAAGAAG CAACTGGCCA GTTCCGGGTA 420

TACCCGGAGC TGCCCAAGCC CTCCATCTCC AGCAACAACT CCAAACCCGT GGAGGACAAG 480

GATGCTGTGG CCTTCACCTG TGAACCTGAG ACTCAGGACG CAACCTACCT GTGGTGGGTA 540

AACAATCAGA GCCTCCCGGT CAGTCCCAGG CTGCAGCTGT CCAATGGCAA CAGGACCCTC 600

ACTCTATTCA ATGTCACAAG AAATGACACA GCAAGCTACA AATGTGAAAC CCAGAACCCA 660

GTGAGTGCCA GGCGCAGTGA TTCAGTCATC CTGAATGTCC TCTATGGCCC GGATGCCCCC 720

ACCATTTCCC CTCTAAACAC ATCTTACAGA TCAGGGGAAA ATCTGAACCT CTCCTGCCAC 780

GCAGCCTCTA ACCCACCTGC ACAGTACTCT TGGTTTGTCA ATGGGACTTT CCAGCAATCC 840

ACCCAAGAGC TCTTTATCCC CAACATCACT GTGAATAATA GTGGATCCTA TACGTGCCAA 900

GCCCATAACT CAGACACTGG CCTCAATAGG ACCACAGTCA CGACGATCAC AGTCTATGCA 960

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GTAGCCTTAA CCTGTGAACC TGAGATTCAG AACACAACCT ACCTGTGGTG GGTAAATAAT 1080

CAGAGCCTCC CGGTCAGTCC CAGGCTGCAG CTGTCCAATG ACAACAGGAC CCTCACTCTA 1140

CTCAGTGTCA CAAGGAATGA TGTAGGACCC TATGAGTGTG GAATCCAGAA CGAATTAAGT 1200

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GAGCTCTTTA TCTCCAACAT CACTGAGAAG AACAGCGGAC TCTATACCTG CCAGGCCAAT 1440

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GTCACAAGAA ATGACGCAAG AGCCTATGTA TGTGGAATCC AGAACTCAGT GAGTGCAAAC 1740

CGCAGTGACC CAGTCACCCT GGATGTCCTC TATGGGCCGG ACACCCCCAT CATTTCCCCC 1800

CCAGACTCGT CTTACCTTTC GGGAGCGAAC CTCAACCTCT CCTGCCACTC GGCCTCTAAC 1860

CCATCCCCGC AGTATTCTTG GCGTATCAAT GGGATACCGC AGCAACACAC ACAAGTTCTC 1920

TTTATCGCCA AAATCACGCC AAATAATAAC GGGACCTATG CCTGTTTTGT CTCTAACTTG 1980

GCTACTGGCC GCAATAATTC CATAGTCAAG AGCATCACAG TCTCTGCATA G 2031

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGCCGCAATA ATTCCATAGT CAAGAGCATC ACAGTCTCTG CATAGT 46

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTAGACTATG CAGAGACTGT GATGCTCTTG ACTATGGAAT TATTGC 46

Claims

1. A recombinant CEA glycoprotein derivative characterized in that it is:
(a) free from cross-reactive CEA-like antigens;
(b) antigenically indistingiushable from the soluble form of CEA shed from tumor cells; and
(c) devoid of ethanolamine.

2. A recombinant CEA glycoprotein derivative according to claim 1, having the amino acid sequence

KLTIESTPFN VAEGKEVLLL VHNLPQHLFG YSWYKGERVD GNRQIIGYVI

GTQQATPGPA YSGREIIYPN ASLLIQNIIQ NDTGFYTLHV IKSDLVNEEA

TGQFRVYPEL PKPSISSNNS KPVEDKDAVA FTCEPETQDA TYLWWVNNQS

LPVSPRLQLS NGNRTLTLFN VTRNDTASYK CETQNPVSAR RSDSVILNVL

YGPDAPTISP LNTSYRSGEN LNLSCHAASN PPAQYSWFVN GTFQQSTQEL

FIPNITVNNS GSYTCQAHNS DTGLNRTTVT TITVYAEPPK PFITSNNSNP

VEDEDAVALT CEPEIQNTTY LWWVNNQSLP VSPRLQLSND NRTLTLLSVT

RNDVGPYECG IQNELSVDHS DPVILNVLYG PDDPTISPSY TYYRPGVNLS

LSCHAASNPP AQYSWLIDGN IQQHTQELFI SNITEKNSGL YTCQANNSAS

GHSRTTVKTI TVSAELPKPS ISSNNSKPVE DKDAVAFTCE PEAQNTTYLW

WVNGQSLPVS PRLQLSNGNR TLTLFNVTRN DARAYVCGIQ NSVSANRSDP

VTLDVLYGPD TPIISPPDSS YLSGANLNLS CHSASNPSPQ YSWRINGIPQ

QHTQVLFIAK ITPNNNGTYA CFVSNLATGR NNSIVKSITV SA **SEQ ID NO: 1]**

or an allelic variant or functional equivalent thereof.

3. A DNA encoding a recombinant CEA glycoprotein derivative according to claim 1 or 2.

4. A DNA encoding a recombinant CEA glycoprotein derivative according to claim 1 or 2 having all or part of the nucleotide sequence

ATGGAGTCTC CCTCGGCCCC TCCCCACAGA TGGTGCATCC CCTGGCAGAG

GCTCCTGCTC ACAGCCTCAC TTCTAACCTT CTGGAACCCG CCCACCACTG

CCAAGCTCAC TATTGAATCC ACGCCGTTCA ATGTCGCAGA GGGGAAGGAG

GTGCTTCTAC TTGTCCACAA TCTGCCCCAG CATCTTTTTG GCTACAGCTG

GTACAAAGGT GAAAGAGTGG ATGGCAACCG TCAAATTATA GGATATGTAA

TAGGAACTCA ACAAGCTACC CCAGGGCCCG CATACAGTGG TCGAGAGATA

ATATACCCCA ATGCATCCCT GCTGATCCAG AACATCATCC AGAATGACAC

AGGATTCTAC ACCCTACACG TCATAAAGTC AGATCTTGTG AATGAAGAAG

CAACTGGCCA GTTCCGGGTA TACCCGGAGC TGCCCAAGCC CTCCATCTCC

AGCAACAACT CCAAACCCGT GGAGGACAAG GATGCTGTGG CCTTCACCTG

TGAACCTGAG ACTCAGGACG CAACCTACCT GTGGTGGGTA AACAATCAGA

GCCTCCCGGT CAGTCCCAGG CTGCAGCTGT CCAATGGCAA CAGGACCCTC

ACTCTATTCA ATGTCACAAG AAATGACACA GCAAGCTACA AATGTGAAAC

CCAGAACCCA GTGAGTGCCA GGCGCAGTGA TTCAGTCATC CTGAATGTCC

TCTATGGCCC GGATGCCCCC ACCATTTCCC CTCTAAACAC ATCTTACAGA

TCAGGGGAAA ATCTGAACCT CTCCTGCCAC GCAGCCTCTA ACCCACCTGC

ACAGTACTCT TGGTTTGTCA ATGGGACTTT CCAGCAATCC ACCCAAGAGC

TCTTTATCCC CAACATCACT GTGAATAATA GTGGATCCTA TACGTGCCAA

GCCCATAACT CAGACACTGG CCTCAATAGG ACCACAGTCA CGACGATCAC

AGTCTATGCA GAGCCACCCA AACCCTTCAT CACCAGCAAC AACTCCAACC

CCGTGGAGGA TGAGGATGCT GTAGCCTTAA CCTGTGAACC TGAGATTCAG

AACACAACCT ACCTGTGGTG GGTAAATAAT CAGAGCCTCC CGGTCAGTCC

CAGGCTGCAG CTGTCCAATG ACAACAGGAC CCTCACTCTA CTCAGTGTCA

CAAGGAATGA TGTAGGACCC TATGAGTGTG GAATCCAGAA CGAATTAAGT

GTTGACCACA GCGACCCAGT CATCCTGAAT GTCCTCTATG GCCCAGACGA

CCCCACCATT TCCCCCTCAT ACACCTATTA CCGTCCAGGG GTGAACCTCA

GCCTCTCCTG CCATGCAGCC TCTAACCCAC CTGCACAGTA TTCTTGGCTG

ATTGATGGGA ACATCCAGCA ACACACACAA GAGCTCTTTA TCTCCAACAT

CACTGAGAAG AACAGCGGAC TCTATACCTG CCAGGCCAAT AACTCAGCCA

GTGGCCACAG CAGGACTACA GTCAAGACAA TCACAGTCTC TGCGGAGCTG

CCCAAGCCCT CCATCTCCAG CAACAACTCC AAACCCGTGG AGGACAAGGA

TGCTGTGGCC TTCACCTGTG AACCTGAGGC TCAGAACACA ACCTACCTGT

GGTGGGTAAA TGGTCAGAGC CTCCCAGTCA GTCCCAGGCT GCAGCTGTCC

AATGGCAACA GGACCCTCAC TCTATTCAAT GTCACAAGAA ATGACGCAAG

AGCCTATGTA TGTGGAATCC AGAACTCAGT GAGTGCAAAC CGCAGTGACC

CAGTCACCCT GGATGTCCTC TATGGGCCGG ACACCCCCAT CATTTCCCCC

CCAGACTCGT CTTACCTTTC GGGAGCGAAC CTCAACCTCT CCTGCCACTC

GGCCTCTAAC CCATCCCCGC AGTATTCTTG GCGTATCAAT GGGATACCGC

AGCAACACAC ACAAGTTCTC TTTATCGCCA AAATCACGCC AAATAATAAC

GGGACCTATG CCTGTTTTGT CTCTAACTTG GCTACTGGCC GCAATAATTC

CATAGTCAAG AGCATCACAG TCTCTGCATAG [SEQ ID NO: 2]

or a functional equivalent thereof.

5. A recombinant vector comprising a DNA having a nucleotide sequence encoding a recombinant CEA glycoprotein derivative according to claim 1 or 2, which recombinant vector is capable of directing the expression of the said DNA in a compatible host cell.

6. A transformed host cell containing a recombinant vector comprising a DNA having a nucleotide sequence encoding a recombinant CEA glycoprotein derivative according to claim 1 or 2, which transformed host cell is capable of expressing the said DNA.

7. A recombinant CEA glycoprotein derivative according to claim 1 or 2 for diagnostic purposes.

8. A reagent for the diagnosis of neoplastic diseases comprising a recombinant CEA glycoprotein derivative as claimed in claim 1 or 2 and an inert carrier material.

9. A process for the production of a recombinant CEA glycoprotein as claimed in claim 1 or 2, , which process comprises:
(a) culturing a host cell containing a recombinant vector comprising a DNA having a nucleotide sequence encoding the said recombinant CEA glycoprotein derivative, such as the DNA having the nucleotide sequence [SEQ ID NO: 2] or an equivalent sequence thereof, under conditions in which the DNA is expressed; and
(b) isolating the recombinant CEA glycoprotein derivative produced by the host cell from the culture.

10. A process for the production of a recombinant vector comprising a DNA having a nucleotide sequence encoding a recombinant CEA glycoprotein as claimed in claim 1 or 2, which process comprises:
(a) inserting a DNA having a nucleotide sequence encoding the recombinant CEA glycoprotein derivative into a vector;
(b) replicating the said vector in a host cell; and
(c) isolating the recombinant vector from the host cell.

11. A method for preparing a reagent for the diagnosis of neoplastic diseases, characterized in that a recombinant CEA glycoprotein derivative as claimed in claim 1 or 2 is mixed with an inert carrier material.

12. A method for diagnosis of a neoplastic disease in a biological sample which method comprises measuring the CEA levels in said sample by using a recombinant CEA glycoprotein derivative according to claim 1 or 2 in an immunoassay.

13. The use of a recombinant CEA glycoprotein derivative as claimed in claim 1 or 2 for determining the presence of tumor cells in a biological sample, e.g. a sample of a body fluid.

14. A recombinant CEA glycoprotein derivative as claimed in claim 1 or 2, whenever prepared by a process as claimed in claim 9.

15. A recombinant vector comprising a DNA having a nucleotide sequence encoding a recombinant CEA glycoprotein as claimed in claim 1 or 2, whenever prepared by a process as claimed in claim 10.

16. A test-kit for the diagnosis of neoplastic diseases, which test-kit comprises in a container a recombinant CEA glycoprotein as claimed in claim 1 or 2, if necessary in combination with an inert carrier material, and if necessary additional reagents such as e.g. a monoclonal or a polyclonal antibody directed against CEA.

17. The invention as hereinbefore described.

Fig. 1/4

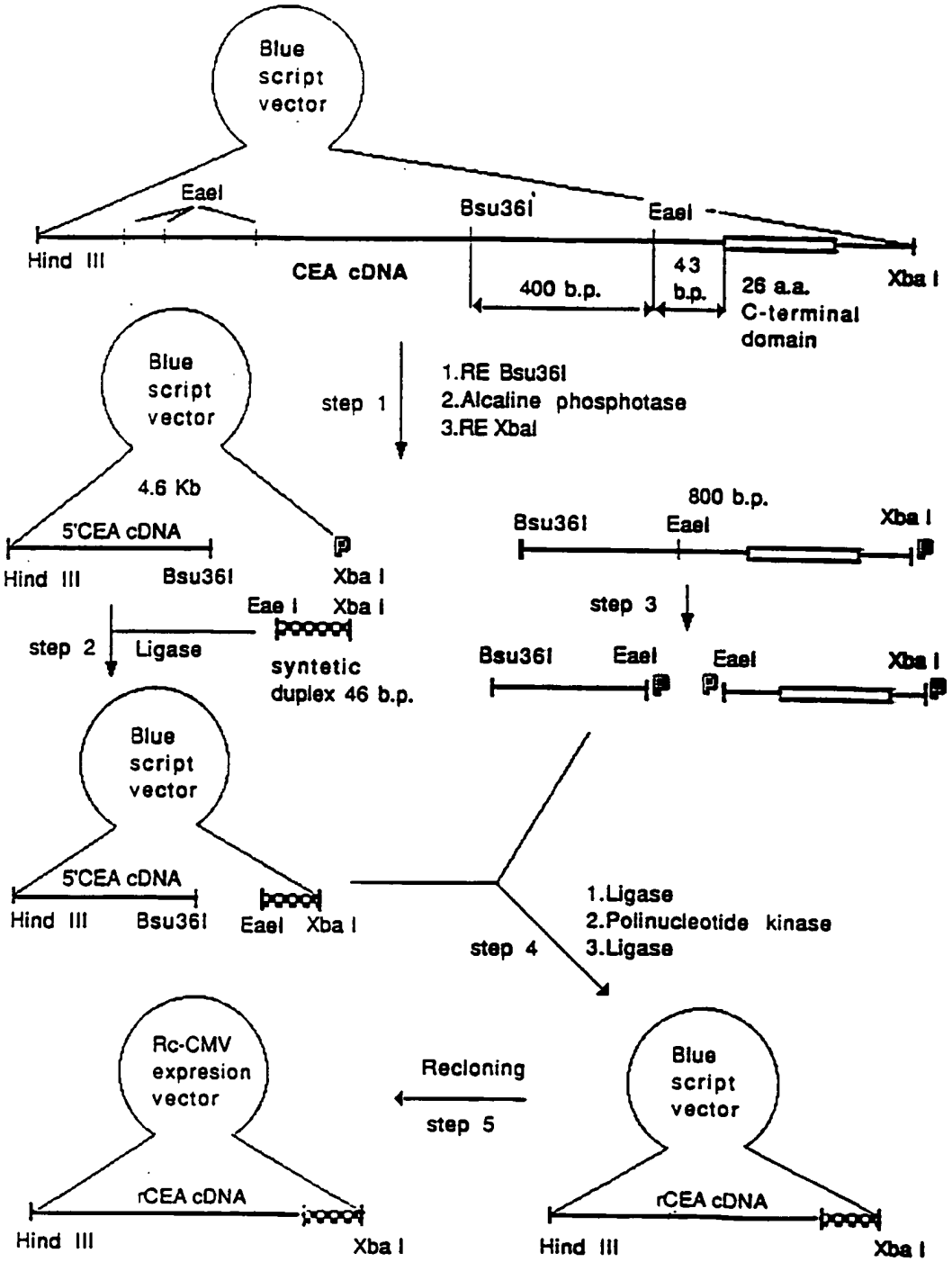

Blue script vector
EaeI
Bsu36I
EaeI
Hind III
CEA cDNA
400 b.p.
43 b.p.
26 a.a. C-terminal domain
Xba I
step 1
1.RE Bsu36I
2.Alcaline phosphotase
3.RE XbaI
4.6 Kb
5'CEA cDNA
Hind III
Bsu36I
Xba I
800 b.p.
Bsu36I
EaeI
Xba I
step 3
Eae I
Xba I
step 2
Ligase
syntetic duplex 46 b.p.
Bsu36I
EaeI
EaeI
Xba I
Blue script vector
5'CEA cDNA
Hind III
Bsu36I
EaeI
Xba I
1.Ligase
2.Polinucleotide kinase
3.Ligase
step 4
Rc-CMV expresion vector
rCEA cDNA
Hind III
Xba I
Recloning
step 5
Blue script vector
rCEA cDNA
Hind III
Xba I

Fig. 2/4

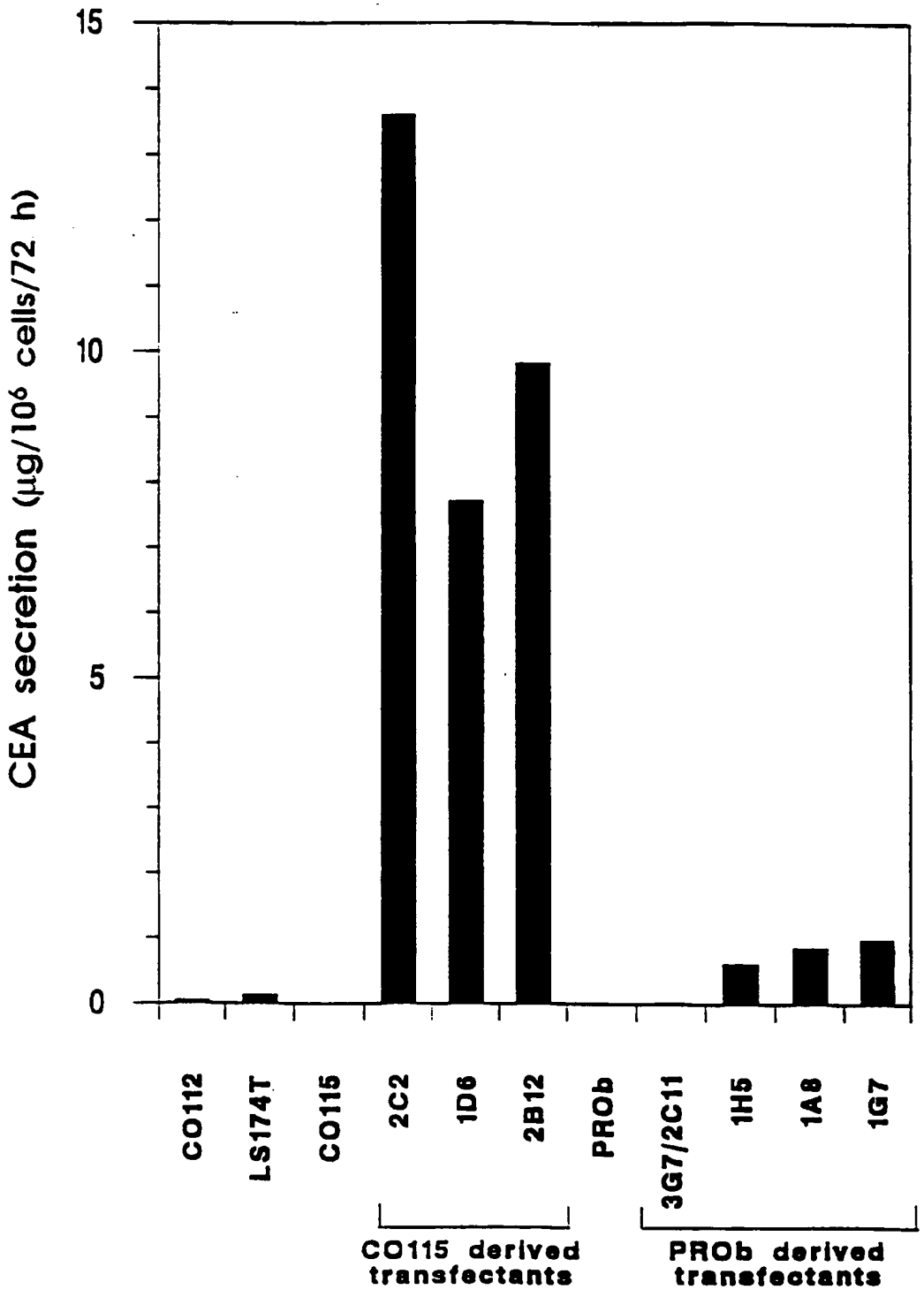

15
10
5
0
CEA secretion (µg/10⁶ cells/72 h)
CO112
LS174T
CO115
2C2
1D6
2B12
PROb
3G7/2C11
1H5
1A8
1G7
CO115 derived transfectants
PROb derived transfectants

Fig. 3/4

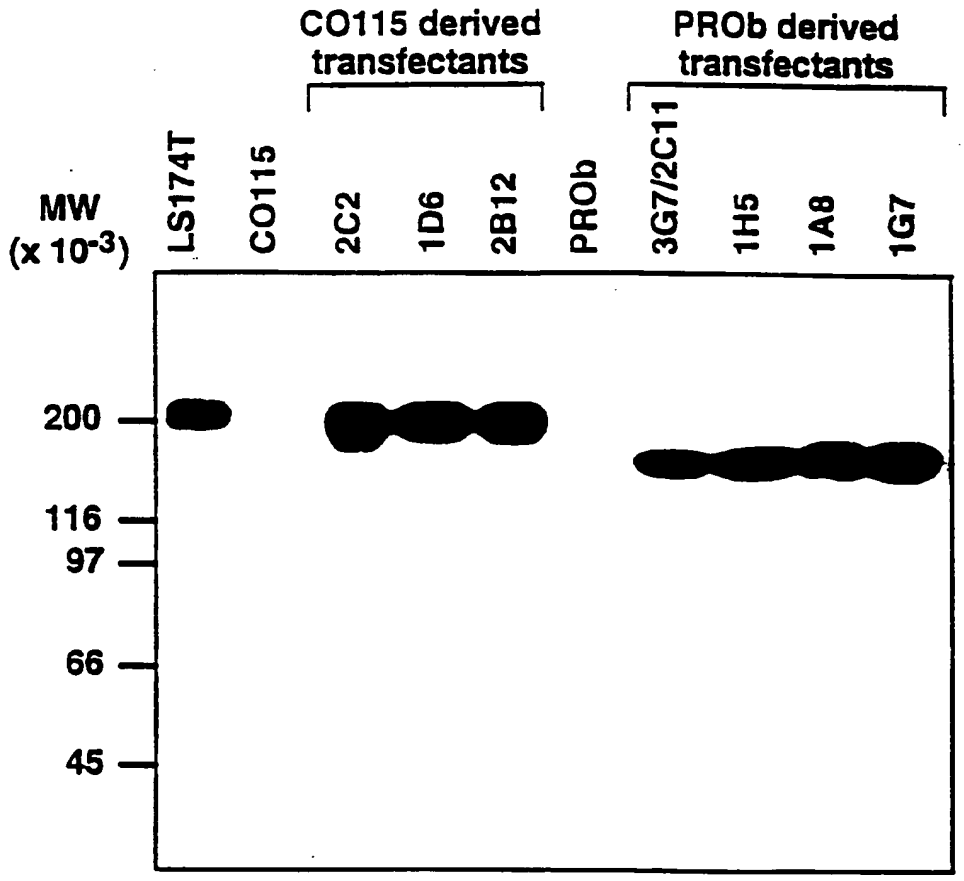
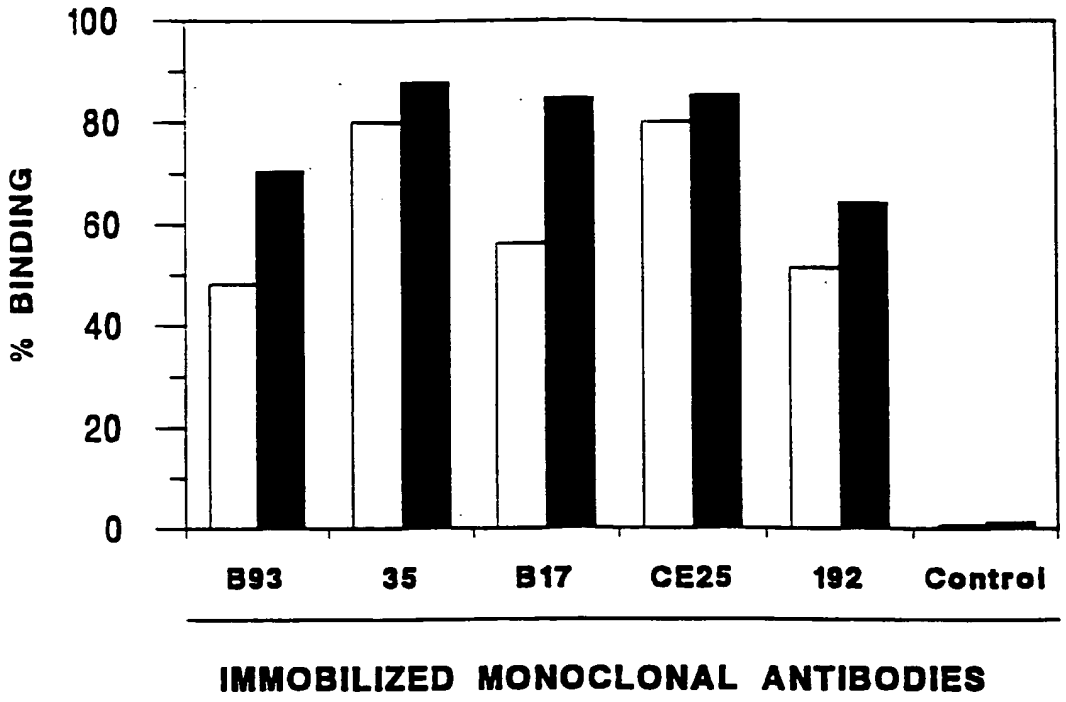

CO115 derived transfectants
PROb derived transfectants
MW (x 10-3)
LS174T
CO115
2C2
1D6
2B12
PROb
3G7/2C11
1H5
1A8
1G7
200
116
97
66
45

Fig. 4/4


100
80
60
40
20
0
% BINDING
B93
35
B17
CE25
192
Control
IMMOBILIZED MONOCLONAL ANTIBODIES

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 10 3986

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 155, no. 2 , 15 September 1988 , DULUTH, MINNESOTA US pages 794 - 800 JEAN F;MALAPERT P;ROUGON G;BARBET J; 'Cell membrane, but not circulating, carcinoembryonic antigen is linked to a phosphatidylinositol-containing hydrophobic domain.' * the whole document * ---	1-17	C12N15/12 C07K13/00 C12N5/10 G01N33/68 C12P21/08
Y	PATENT ABSTRACTS OF JAPAN vol. 12, no. 450 (C-547)25 November 1988 & JP-A-63 177 794 (SUNTORY LTD.) 21 July 1988 * abstract * ---	1-17	
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 85, no. 13 , July 1988 , WASHINGTON US pages 4648 - 4652 HEFTA SA;HEFTA LJ;LEE TD;PAXTON RJ;SHIVELY JE; 'Carcinoembryonic antigen is anchored to membranes by covalent attachment to a glycosylphosphatidylinositol moiety: identification of the ethanolamine linkage site.' * the whole document * ---	1-17	
Y	EP-A-0 263 933 (MOLECULAR DIAGNOSTICS, INC.; US) 20 April 1988 * the whole document * ---	1-17	
	-/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.5)
C12N C07K G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	29 June 1994	Nauche, S

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 10 3986

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Y	IMMUNOLOGICAL INVESTIGATIONS vol. 21, no. 3 , 1992 pages 241 - 257 KUROKI, M. ET AL.; 'Immunoreactivity of recombinant carcinoembryonic antigen proteins expressed in Esherichia Coli' * the whole document * ---	1-17	
A	FR-A-2 095 728 (F. HOFFMANN LA ROCHE & CIE, CH) 11 February 1972 ---		
P,X	MOLECULAR IMMUNOLOGY vol. 30, no. 10 , July 1993 pages 921 - 927 TERSKIKH A;MACH JP;PELEGRIN A; 'Marked increase in the secretion of a fully antigenic recombinant carcinoembryonic antigen obtained by deletion of its hydrophobic tail.' * the whole document * ---	1-17	
Y	EP-A-0 346 710 (MOLECULAR DIAGNOSTICS, INC;US) 20 December 1989 * the whole document * -----	1-17	TECHNICAL FIELDS SEARCHED (Int.Cl.5)

The present search report has been drawn up for all claims

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P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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